CANCER IMMUNOTHERAPY WITH A VIRAL ANTIGEN-DEFINED, IMMUNOMODULATOR-SECRETING CELL VACCINE

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

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FIELD OF THE INVENTION

[0002] The present invention relates to cancer immunotherapy and cancer vaccine development.

BACKGROUND OF THE INVENTION

[0003] Despite ongoing efforts to define immunologically relevant tumor antigens, very little is known about most tumor rejection antigens for the majority of human cancers. Consequently, most cancer vaccine approaches currently use tumor cells as a source of antigen. Early generations of cell-based cancer vaccines have consisted of killed tumor cells or tumor cell lysates mixed with adjuvants, such as *Bacillus Calmette Guerin* (BCG) and *Corynebacterium parvum*, in an attempt to amplify tumor-specific immune responses (Berd et al., J. Clin. Oncol. 8: 1858-1867 (1990)). Subsequently, genetically modified tumor vaccines replaced the most complex and inconsistent mixtures of tumor cells and bacteria. Currently, the most popular genetically modified cell-based vaccines take advantage of the large set of cloned genes encoding cytokines and co-stimulatory molecules (Pardoll, Ann. Rev. Immunol. 13: 399-415 (1995)).

[0004] Among the different cytokines used to modify tumor immunogenicity, granulocyte-macrophage colony stimulating factor (GM-CSF) appears to be the most potent (Dranoff et al., PNAS USA 90: 3539-3543 (1993)). GM-CSF induces the diffferentiation of primitive hematopoietic precursors into dendritic cells (DC), a type of antigen-presenting cell (APC) that initiates the most potent T-cell responses (Banchereau et al., Nature 392 (6673): 245-252 (1998)) and promotes DC recruitment and differentiation at the site of vaccination. Thus, DC play a central role in priming immunological response.

[0005] GM-CSF-secreting cellular vaccines have been shown to eradicate small, pre-established tumors in mice (Dranoff et al., *supra*; and Levitsky et al., J. Immunol. 156: 3858-3865 (1996)). Furthermore, promising results have been obtained in human patients afflicted with melanoma (Soiffer et al., PNAS USA 95: 13141-13146 (1998)), prostate and renal cell carcinoma (Simons et al., Cancer Res. 59: 5160-5168 (1999); and Simons et al., Cancer Res. 57: 1537-1546 (1997)), and pancreatic cancer (Jaffee et al., J. Clin. Oncol. 19: 145-156 (2001)). These trials consistently demonstrated systemic anti-tumor immunity in patients and suggest an improvement in overall survival in those patients in whom evidence of vaccine efficacy was demonstrated by the development of tumor-specific delayed type hypersensitivity (DTH) responses (Jaffee et al., *supra*).

[0006] Unfortunately, modification of autologous tumor cells to express a cytokine, such as GM-CSF, is highly individualized, expensive, and labor-intensive. Therefore, simpler approaches that maintain the immunological activity of paracrine cytokine production have been developed. One such approach utilizes a universal bystander cell line altered to produce a large and stable amount of GM-CSF (see, e.g., Levitsky et al., U.S. Pat. No. 6,464,973 and Int'l Pat. App. No. PCT/US99/02253) in combination with an antigen of the cancer to be treated, such as, for example, tumor cells isolated from the patient (Borrello et al., Hum. Gene Ther. 10: 1983-1991 (1999); Borrello et al., Blood 95: 3011-3019 (2000)). This approach obviates the need for *in vitro* passaging or modification, such as by transduction, of each patient's tumor cells, thereby guaranteeing a constant amount of cytokine production without any intra- or inter-patient variability, while utilizing the patient-specific antigenic repertoire. An allogeneic, GM-CSF-secreting human erythroleukemia cell line, namely K562, is currently being used in two phase I trials at Johns Hopkins University for vaccination of multiple myeloma and acute myelogenous leukemia (AML), in combination with irradiated autologous tumor cells.

[0007] Vaccination of mice afflicted with lymphoma with a mixture of autologous tumor cells and GM-CSF-producing MHC class I- and MHC class II-negative cells, namely B78H1/GM-CSF cells, primed an anti-tumor immune response. The anti-tumor immune response was equivalent to or better than those achieved using autologous tumor cells directly transduced to secrete GM-CSF.

[0008] GM-CSF-secreting cellular vaccines, which are currently in use, are not specific for a defined tumor antigen. Hence, it is not possible to target such vaccines and evaluate fully their anti-tumor immune responses. It is an object of the present invention, therefore, to provide a GM-CSF-secreting cellular vaccine that is specific for a defined tumor antigen. Such a vaccine will enable one to evaluate more fully anti-tumor immune responses. This

and other objects and advantages of the present invention, as well as additional inventive features, will become apparent from the detailed description provided herein.

BRIEF SUMMARY OF THE INVENTION

[0009] The present invention provides a human cell line, which lacks major histocompatibility class I (MHC-I) antigens and major histocompatibility class II (MHC-II) antigens and which has been modified to comprise and express (i) a nucleotide sequence encoding an immunomodulator and (ii) a nucleotide sequence encoding an antigen of Epstein-Barr virus (EBV). Further provided is a composition comprising a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an immunomodulator, and a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of EBV. Still further provided is a composition comprising an immunomodulator and a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of EBV.

[0010] Also provided by the present invention is a method of inducing or stimulating an immune response in a human to an EBV-associated cancer. The method comprises administering to the human the aforementioned human cell line or one of the aforementioned compositions in an amount sufficient to induce or stimulate an immune response to the antigen of EBV expressed by the human cell line, whereupon an immune response to the EBV-associated cancer is induced. Alternatively, a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an immunomodulator, and a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of EBV, can be administered, simultaneously or sequentially in either order, by the same or different routes, to the human in amounts sufficient to induce or stimulate an immune response to an EBV-associated cancer. Also, alternatively, an immunomodulator and a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of EBV, can be administered, simultaneously or sequentially in either order, by the same or different routes, to the human in amounts sufficient to induce or stimulate an immune response to an EBV-associated cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Fig. 1 is a diagram of a bi-cistronic plasmid (arrows indicate direction of transcription) containing the human GM-CSF gene (GM-CSF) operably linked to the CMV promoter (CMV) and a polyA tail (polyA), a hygromycin resistance gene (Hygro^r), an EBV EBNA1 gene (EBNA1), an EBV origin of replication (OriP), and an ampicillin resistance gene (Amp^r). The plasmid is designated pCEP4-EBNA1/hGM-CSF.

[0012] Fig. 2 is a diagram of a bi-cistronic plasmid (arrows indicate direction of transcription) containing the LMP2 coding sequence (LMP2) operably linked to the CMV promoter (CMV) and a polyA tail (polyA), a neomycin resistance gene (Neomycin), and an ampicillin resistance gene (Amp^r). The plasmid is designated pcDNA3-LMP2.

[0013] Fig. 3 is a compilation of various nucleotide (genomic, mRNA, cDNA; etc.) sequences that can be used in the context of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0014] The present invention provides a human cell line, which lacks MHC-I antigens and MHC-II antigens and which has been modified to comprise and express (i) a nucleotide sequence encoding an immunomodulator and (ii) a nucleotide sequence encoding a viral antigen, in particular an antigen of a virus associated with a disease, such as cancer, e.g., EBV, human papilloma virus (HPV), or Kaposi sarcoma herpes virus (KSHV; also known as human herpes virus 8 (HHV8)). The human cell line can be any suitable cell line. Theoretically, any human cell line that is capable of paracrine production of an immunomodulator can be used. The capacity for paracrine production of an immunomodulator is not required when the cell line will not be used to express an immunomodulator in accordance with certain embodiments of the present invention as set forth herein.

[0015] The cell line can naturally lack MHC-I and MHC-II antigens or it can be manipulated or modified so that it does not express MHC-I and MHC-II antigens. In this regard, it will be understood by one of ordinary skill in the art that a cell line is deemed to lack MHC-I and MHC-II antigens if it does not constitutively express such antigens under normal biological conditions. However, a cell line that does not constitutively express MHC-I and MHC-II antigens can, under certain artificially created conditions, such as conditions that can be created *in vitro*, be induced to express MHC-I and/or MHC-II antigens. Such cell lines are considered to lack MHC-I and MHC-II antigens for purposes of the present invention. Likewise, cells having inactivated MHC antigens are also considered to lack such antigens for purposes of the present invention.

[0016] The cell line preferably grows in defined medium. One of ordinary skill in the art appreciates that defined medium is medium, the composition of which is known. In other words, the identity and amount of each and every component of the medium is known. Defined medium does not contain serum inasmuch as the composition of serum is undefined. Preferably, the cell line grows as a suspension.

[0017] A preferred human cell line for modification in accordance with the present invention is K562, which is deposited with the American Type Culture Collection (ATCC) as CCL-243. The K562 cell line is described by Lozzio et al., Blood 45(3): 321-334 (1975), and Klein et al., Int. J. Cancer 18: 421-431 (1976). Other suitable human cell lines include, but are not limited to, SK-MEL-33 (Wang et al., J. Clin. Invest. 91: 684-692 (1993)) and various melanoma cell lines (Ferrone et al., Immunol. Today 16(10): 487-494 (1995); K ageshita et al., Cancer Res. 53(14): 3349-3354 (1993); and Wang et al., Tissue Antigens 47(5): 382-390 (1996)).

[0018] A human cell line that expresses MHC-I antigens can be modified so that it does not express such antigens in any of a number of different ways. For example, one can interfere with the expression and/or transport of the α chain. A human cell line that expresses MHC-II antigens also can be modified in various ways so that it does not express such antigens. For example, one can interfere with the expression and/or transport of the α chains and the β chains. MHC-I and -II antigens also can be inactivated for purposes of the present invention. This can be accomplished in a variety of ways (see, for example, U.S. Patent No. 5,574,205). For example, a "dominant negative" can be created. A single modified β_2 microglobulin gene, whose protein product effectively complexes with MHC-I molecules and acts as a decoy, thereby preventing the insertion of MHC-I antigens into the membrane, can be overexpressed. A similar approach can be used with respect to MHC-II antigens by overexpressing modified genes encoding defective α or β subunits that complex with the host cells' subunits, thereby rendering them nonfunctional. Transfection, retroviral infection or homologous recombination can be used to achieve expression of modified MHC or β_2 microglobulin genes or inactivation of genes.

[0019] Levels of MHC-I antigen on the cell surface can be reduced by introducing into cells a sequence encoding adenoviral E19 protein by transfection or retroviral infection. The protein forms complexes specifically with MHC-I antigens in the rough endoplasmic reticulum preventing normal transport of MHC-I molecules to the plasma membrane (Andersson et al., Cell 43: 215-222 (1985); and Pabo et al., Advances in Cancer Research 42: 151-163 (1989)).

[0020] In addition to lacking MHC-I and MHC-II antigens, the human cell line is modified to comprise and express a nucleotide sequence encoding an immunomodulator and

a nucleotide sequence encoding a viral antigen. By "modified" is meant the introduction into the cell line of a nucleic acid molecule, e.g., a vector, comprising a nucleotide sequence encoding a gene product, which, in the context of the present inventive cell line, is an immunomodulator or an antigen, such as an antigen of EBV, HPV, or KSHV, in operable linkage with a promoter and, as required for expression, various other regulatory sequences. Either the immunomodulator is not expressed in the cell line or, as a result of the introduction of the nucleic acid molecule is now expressed at a greater level.

[0021] A "vector" encompasses a nucleic acid molecule, such as a plasmid, virus or other vehicle, which contains one or more heterologous or recombinant nucleotide sequences, e.g., a nucleotide sequence encoding an immunomodulator and/or a nucleotide sequence encoding an antigen of EBV, HPV or KSHV, wherein the nucleotide sequences can be under the control of the same or different functional promoters, alone or in further combination with enhancer(s), and that is capable of functioning as a vector as that term is understood by those of ordinary skill in the art.

[0022] Any suitable vector can be employed that is appropriate for introduction of nucleic acids into eukaryotic cells, or more particularly animal cells, such as mammalian, e.g., human, cells. Preferably, the vector is compatible with the cell, e.g., can impart expression of the immunomodulator and/or viral antigen, and is stably maintained or relatively stably maintained in the cell. Desirably, the vector comprises an origin of replication. When an immunomodulator coding sequence or viral antigen coding sequence is transferred (i.e., as opposed to an immunomodulator gene having its own promoter or a viral antigen gene having its own promoter), optimally the vector also contains a promoter that can drive expression of the coding sequence and that is operably linked to the coding sequence. A coding sequence is "operably linked" to a promoter (e.g., when both the coding sequence and the promoter together constitute a native or recombinant immunomodulator gene or viral antigen gene) when the promoter can direct transcription of the coding sequence.

[0023] Appropriate viral vectors include, but are not limited to simian virus 40, bovine papilloma virus, Epstein-Barr virus, adenovirus, herpes virus, vaccinia virus, Moloney murine leukemia virus, Harvey murine sarcoma virus, murine mammary tumor virus, and Rous sarcoma virus. Any plasmid suitable for use in a eukaryote, in particular a mammal, e.g., a human, can be used in the context of the present invention. Desirably, the plasmid comprises a promoter, such as the cytomegalovirus promoter, an origin of replication, such as the SV40 origin of replication, a selectable marker, such as antibiotic resistance, and provides for mRNA with poly A tails. A preferred example of a plasmid is pCEP4 (See Examples 1 and 3).

[0024] Reference to a vector or other DNA sequences as "recombinant" merely acknowledges the linkage of DNA sequences, which are not typically conjoined as isolated from nature. A "gene" is any nucleic acid sequence coding for a protein or a nascent mRNA molecule. Whereas a gene comprises coding sequences and non-coding (e.g., regulatory) sequences, a "coding sequence" does not include any non-coding DNA. As used herein, "gene" or "coding sequence" includes genomic or cDNA sequences, greater and lesser sequences and mutations thereof, whether isolated from nature or synthesized in whole or in part, as long as the gene or coding sequence can express a protein having the characteristic function of the immunomodulator, i.e., the ability to stimulate the host immune response, or the characteristic antigenicity of an antigen of a virus. The means of modifying genes or coding sequences are well-known in the art, and also can be accomplished by means of commercially available kits (e.g., New England Biolabs, Inc., Beverly, MA; Clontech, Palo Alto, CA).

[0025] A "promoter" is a DNA sequence that directs the binding of RNA polymerase and thereby promotes RNA synthesis. "Enhancers" are *cis*-acting elements of DNA that stimulate or inhibit transcription of adjacent genes. An enhancer that inhibits transcription also is termed a "silencer." Enhancers differ from DNA-binding sites for sequence-specific DNA binding proteins found only in the promoter (which also are termed "promoter elements") in that enhancers can function in either orientation, and over distances of up to several kilobase pairs (kb), even from a position downstream of a transcribed region.

[0026] The "immunomodulator" can be any suitable immunomodulator, such as a cytokine, a chemokine or an adjuvant, for example, obtained from any suitable source, such as a mammal, e.g., a human. Desirably, the immunomodulator induces or stimulates an immune response to the viral antigen expressed by the cell line. Cell-targeting means also can be considered immunomodulators. Likewise, antibodies (or antigenically reactive fragments thereof), antisense molecules, dsRNAi, and the like also can be considered immunomodulators to the extent that they inhibit or block the ability of a viral gene product to block the action of an interferon, if so desired. For example, the EBNA-2 protein of EBV blocks signal transduction of interferons, the EBER RNA of EBV blocks activation of Pkr, and BCRF1 of EBV is an IL-10 homolog that inhibits IFN-γ, IL-1, IL-2, and TNF synthesis.

[0027] Examples of suitable immunomodulatory cytokines include interferons (e.g., IFNα, IFNβ and IFNγ), interleukins (e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 and IL-20), tumor necrosis factors (e.g., TNFα and TNFβ), erythropoietin (EPO), FLT-3 ligand, gIp10, TCA-3, MCP-1, MIF, MIP-1α, MIP-1β, Rantes, macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), and granulocyte-macrophage colony stimulating factor (GM-CSF), as well as functional

fragments of any of the foregoing. The most preferred immunomodulatory cytokine is GM-CSF, such as human GM-CSF, including a functional fragment thereof. An alternatively preferred immunomodulatory cytokine is IL-2 or a functional fragment thereof. Any immunomodulatory chemokine that binds to a chemokine receptor, i.e., a CXC, CC, C, or CX3C chemokine receptor, can be used in the context of the present invention. Examples of chemokines include, but are not limited to, Mip1α, Mip-1β, Mip-3α (Larc), Mip-3β, Rantes, Hcc-1, Mpif-1, Mpif-2, Mcp-1, Mcp-2, Mcp-3, Mcp-4, Mcp-5, Eotaxin, Tarc, Elc, I309, IL-8, Gcp-2 Gro-α, Gro-β, Gro-γ, Nap-2, Ena-78, Gcp-2, Ip-10, Mig, I-Tac, Sdf-1, and Bca-1 (Blc), as well as functional fragments of any of the foregoing. Examples of adjuvants include, but are not limited to, heat shock protein, CpG, *Listeria monocytogenes*, aluminum hydroxide (for use with soluble antigen), aluminum phosphate (alum; for use with soluble antigen), muramyl dipeptide, muramyl tripeptide, *Mycobacterium tuberculosis*, QuilA (a purified saponin from the plant *Quillaja saponaria*), alone or in further combination with glycosides, cholesterol, and/or phospholids, empty adenoviral capsids; etc. One of ordinary skill in the art will appreciate that some of these adjuvants cannot be expressed from a vector, in which case the adjuvant, when used, in combination with one or more cell lines as described herein, is administered simultaneously or sequentially, in any order, with the one or more cell lines. Preferably, the adjuvant is administered with the viral-antigen-expressing cell line or the defined viral antigen, itself. Preferably, however, the immunomodulator nucleotide sequence encodes a GM-CSF sequence, particularly a human GM-CSF gene or coding sequence, including a human GM-CSF cDNA (e.g., as described by Cantrell et al., PNAS USA 82: 6250-6254 (1985)) or genomic sequence (e.g., as described by Miyatake et al., EMBO J. 4(10): 2561-2568 (1985)).

[0028] The viral antigen can be any defined antigen of a virus that is associated with a disease, such as cancer, in a human. For example, the viral antigen can be any defined antigen of an oncogenic virus. Oncogenic viruses include, but are not limited to, RNA viruses, such as Flaviviridae and Retroviridae, and DNA viruses, such as Hepadnaviridae, Papovaviridae, specifically papillomaviruses, Adenoviridae, Herpesviridae, and Poxviridae. Desirably, the viral antigen is one to which an immune response can be induced or stimulated in a human and is universally recognized. Preferably, the antigen is from EBV, HPV, or KSHV. Examples of suitable EBV antigens for expression in a human cell line in accordance with the present invention are described, for example, in Herbst et al., PNAS USA 88: 4766-4770 (1991). Preferred antigens of EBV include, but are not limited to, Epstein-Barr nuclear antigen-1 (EBNA1), latent membrane protein 1 (LMP1), or latent membrane protein 2 (LMP2). LMP2 is an especially preferred antigen of EBV. A cell line that expresses an antigen of EBV, in particular EBNA1, LMP1 or LMP2, can be used to

induce or stimulate an immune response in a human to an EBV-associated disease or cancer. In the event that immune responses are to be measured in accordance with methods set forth herein, preferably the EBV antigen is one that results in a CD8+ T-cell response that can be readily/easily measured. Examples of suitable HPV antigens for expression in a human cell line in accordance with the present invention are described, for example, in Van Ranst et al.; Virology 190(2): 587-596 (1992); and Rho et al.; Virology 203(1): 158-161 (1994). Preferred antigens of HPV include, but are not limited, E5, E6, and E7. Examples of suitable KSHV antigens for expression in a human cell line in accordance with the present invention are described, for example, in Russo et al., PNAS USA 93(25): 14862-14867 (1996). Preferred antigens of KSHV include, but are not limited to, latency nuclear antigen (LANA) and v-cyclin.

[0029] Preferably, all proper transcription, translation and processing signals (e.g., splicing and polyadenylation signals) are correctly arranged on the vector, such that the immunomodulator (viral antigen) nucleotide sequence will be appropriately transcribed and translated in the cell into which it is introduced. The manipulation of such signals to ensure appropriate expression in host cells is well within the knowledge and expertise of the ordinary skilled artisan. Whereas an immunomodulator gene is controlled by (i.e., operably linked to) its own promoter, another promoter, including a constitutive promoter, such as, for instance the adenoviral type 2 (Ad2) or type 5 (Ad5) major late promoter (MLP) and tripartite leader, the cytomegalovirus (CMV) immediate early promoter/enhancer, the Rous sarcoma virus long terminal repeat (RSV-LTR), and others, can be employed to command expression of the immunomodulator coding sequence. The CMV promoter is a preferred promoter. The same can also be said for the viral antigen gene.

[0030] Alternately, a tissue-specific promoter (i.e., a promoter that is preferentially activated in a given tissue and results in expression of a gene product in the tissue where activated) can be used in the vector. Such promoters include, but are not limited to, the elastase I gene control region, which is active in pancreatic acinar cells as described by Swift et al., Cell 38: 639-646 (1984) and MacDonald, Hepatology 7: 425-515 (1987); the insulin gene control region, which is active in pancreatic beta cells as described by Hanahan, Nature 315: 115-122 (1985); the hepatocyte-specific promoter for albumin or α_1-antitrypsin described by Frain et al., Mol. Cell. Biol. 10: 991-999 (1990), and Ciliberto et al., Cell 41: 531-540 (1985); and the albumin and alpha$_1$-antitrypsin gene control regions, which are both active in liver as described by Pinkert et al., Genes and Devel. 1: 268-276 (1987), and Kelsey et al., Genes and Devel. 1: 161-171 (1987).

[0031] Similarly, a tumor-specific promoter, such as the carcinoembryonic antigen for colon carcinoma described by Schrewe et al., Mol. Cell Biol. 10: 2738-2748 (1990), can be

used in the vector. Along the same lines, promoters that are selectively activated at different developmental stages (e.g., globin genes are differentially transcribed in embryos and adults) can be employed for gene therapy of certain types of cancer.

[0032] Another option is to use an inducible promoter, such as the IL-8 promoter, which is responsive to TNF, or the 6-16 promoter, which is responsive to interferons, or to use other similar promoters responsive to other cytokines or other factors present in a host or that can be administered exogenously. Use of a cytokine-inducible promoter has the added advantage of allowing for auto-inducible expression of a cytokine gene. According to the invention, any promoter can be altered by mutagenesis, so long as it has the desired binding capability and promoter strength.

[0033] Various methods can be employed for delivering a nucleic acid molecule, e.g., a vector, to a cell *in vitro*. For instance, such methods include electroporation, membrane fusion with liposomes, high velocity bombardment with DNA-coated microprojectiles, incubation with calcium phosphate-DNA precipitate, DEAE-dextran mediated transfection, infection with modified viral nucleic acids, direct microinjection into single cells, and the like. Other methods are available and are known to those skilled in the art. The immunomodulator and the EBV antigen can be encoded on the same or different nucleic acid molecules.

[0034] If the cell line is to be used in the context of cancer immunotherapy, the immunomodulator desirably is one that induces or stimulates an immune response against a cancer cell or a cancer antigen, i.e., any protein, carbohydrate or other component that can elicit an immune response, in particular, a defined viral antigen as expressed by a cell line in accordance with the present invention. An inhibitory cytokine or a cytokine that prevents priming cannot be used in the context of cancer immunotherapy. While the nucleic acid molecule preferably encodes a single immunomodulator, the nucleic acid molecule can encode two or more immunomodulators, which can be of the same type, e.g., both cytokines, such as cytokines that act synergistically, or of different types, e.g., a cytokine and an adjuvant.

[0035] For purposes of identification and selection, preferably the nucleic acid molecule comprising a nucleotide sequence encoding an immunomodulator operably linked to a promoter and/or a nucleotide sequence encoding a viral antigen operably linked to a promoter further comprises a nucleotide sequence encoding a selectable marker operably linked to a promoter. In other words, the nucleotide sequence encoding the immunomodulator, the nucleotide sequence encoding the viral antigen, and the nucleotide sequence encoding the selectable marker can be on the same nucleic acid molecule or on

different nucleic acid molecules in various combinations. Likewise, the nucleotide sequences can be under the control of the same or different promoters.

[0036] Preferably, the selectable marker is an antibiotic resistance gene, such as hygromycin resistance. When the selectable marker is hygromycin resistance, preferably the cell line is selected by growth in a culture medium comprising at least about 400 μg/ml hygromycin, more preferably at least about 1,000 μg/ml hygromycin.

[0037] A composition or implant, either one of which comprises an above-described cell line and which is appropriate for administration *in vivo*, can comprise appropriate carriers or diluents, which further can be pharmaceutically acceptable. The means of making such a composition or an implant have been described in the art, see, for instance, *Remington's Pharmaceutical Sciences*, 16th Ed., Mack, ed. (1980). Use of a balanced salt solution, such as Hanks' balanced salt solution, is preferred in the composition.

[0038] Alternatively, the composition can comprise a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an immunomodulator, and a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of a virus that causes disease or cancer, such as an antigen of EBV, HPV or KSHV. Also, alternatively, the composition can comprise an immunomodulator and a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of a virus that causes disease or cancer, such as an antigen of EBV, HPV or KSHV.

[0039] In pharmaceutical dosage form, a composition can be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds as are known in the art.

[0040] A composition of the present invention can be provided in unit dosage form, wherein each dosage unit contains a predetermined amount of the composition, alone or in appropriate combination with other active agents. The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and other mammalian subjects, each unit containing a predetermined quantity of the composition of the present invention, alone or in combination with another active agent, calculated in an amount sufficient to produce the desired effect, in association with a pharmaceutically acceptable diluent, carrier, or vehicle, where appropriate. The specifications for the novel unit dosage forms of the present invention depend on the particular pharmacodynamics associated with the pharmaceutical composition in the particular host.

[0041] In view of the foregoing, the present invention also provides a method of inducing or stimulating an immune response in a human to a virus associated with a disease,

such as cancer. The induction or stimulation of an immune response can be prophylactic or therapeutic and use of the phrase "inducing or stimulating" is intended to cover prophylactic and therapeutic embodiments. For example, evidence is emerging that humans who have had infectious mononucleosis are at risk for developing Hodgkin's disease. Thus, the method of the present invention can be used to inhibit the onset of a virus-associated disease or virus-associated cancer/malignancy. In this regard, one of ordinary skill in the art will appreciate that, while prevention is desirable, "prophylactic" means any degree in the inhibition of the onset of virus-associated disease or virus-associated cancer inasmuch as any inhibition is beneficial. Likewise, one of ordinary skill in the art will appreciate that, while cure is desirable, "therapeutic" means any degree of inhibition/treatment of virus-associated disease or virus-associated cancer, ranging from no change in the disease or cancer, which can be beneficial inasmuch as the disease or cancer does not get worse, to a lessening or an improvement of the disease or a reduction in cancer (size of a tumor and/or number of tumor) or an inhibition of metastasis of the cancer.

[0042] In particular, the present invention provides a method of inducing or stimulating an immune response to an EBV-associated disease or cancer, in particular an EBV-associated cancer. Likewise, the present invention provides a method of inducing or stimulating an immune response to a KSHV-associated disease or cancer, such as a KSHV-associated cancer, and a method of inducing or stimulating an immune response to an HPV-associated disease or cancer, in particular an HPV-associated cancer. The method comprises administering to the human an above-described human cell line in an amount sufficient to induce or stimulate an immune response to the virus-associated disease or cancer, e.g., malignancy. Upon administration of the cell line, an immune response to the virus-associated disease or cancer, e.g., malignancy, is induced or stimulated.

[0043] Alternatively, a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an immunomodulator, and a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of EBV, can be administered, simultaneously or sequentially in either order, by the same or different routes, to the human in amounts sufficient to induce or stimulate an immune response to an EBV-associated cancer. Also, alternatively, an immunomodulator and a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of EBV, can be administered, simultaneously or sequentially in either order, by the same or different routes, to the human in amounts sufficient to induce or stimulate an immune response to an EBV-associated cancer.

[0044] Examples of EBV-associated cancers/malignancies include Burkitt's lymphoma, T-cell lymphoma, nasopharyngeal carcinoma, Hodgkin's lymphoma, B-cell lymphoma, gastric carcinoma, parotid carcinoma, breast carcinoma, and leiomyosarcoma. An example of an HPV-associated cancer/malignancy is cervical cancer. KSHV is associated with Kaposi's sarcoma, for example.

[0045] "Administering" means the actual physical introduction of the composition into or onto (as appropriate) the host. Any and all methods of introducing the composition into the host are contemplated according to the invention; the method is not dependent on any particular means of introduction and is not to be so construed. Means of introduction are well-known to those skilled in the art, and also are exemplified herein.

[0046] Any suitable route of administration can be used. Preferably, the composition is administered subcutaneously or intratumorally. One skilled in the art will recognize that, although more than one route can be used for administration, a particular route can provide a more immediate and more effective reaction than another route. Local or systemic delivery can be accomplished by administration comprising application or instillation of the formulation into body cavities, inhalation or insufflation of an aerosol, or by parenteral introduction, comprising intramuscular, intravenous, intraportal, intrahepatic, peritoneal, subcutaneous, or intradermal administration. In the event that the tumor is in the central nervous system, the composition must be administered intratumorally because there is no priming of the immune system in the central nervous system.

[0047] The amount of cells to be administered to induce or stimulate an immune response to the defined viral antigen can be determined empirically (see, also, Examples 2 and 4 herein). For example, an initial low dosage of cells can be administered and the immune response to the defined viral antigen can be measured. If no immune response is induced or stimulated or it is deemed to be too low, the dosage of cells can be increased. This process can be repeated every week or two weeks or so until an effective dosage is administered.

[0048] One skilled in the art also is aware of means to monitor a therapeutic (i.e., systemic immune) response upon administering a composition of the present invention. In particular, the therapeutic response can be assessed by monitoring attenuation of tumor growth and/or tumor regression. The attenuation of tumor growth or tumor regression in response to treatment can be monitored using several end-points known to those skilled in the art including, for instance, number of tumors, tumor mass or size, or reduction/prevention of metastasis. Methods of assessing cervical cancer are described, for example, in U.S. Pat. No. 6,388,064. These described methods are by no means all-

inclusive, and further methods to suit the specific application will be apparent to the ordinary skilled artisan.

[0049] Any type of viral-associated cancer can be treated in accordance with the present inventive method as long as an antigen of the virus associated with the cancer has been defined and, desirably, is present on the surfaces of the cancerous cells. "Cancer" as used herein includes cancers, in particular those of epithelial origin, characterized by abnormal cellular proliferation and the absence of contact inhibition, which can be evidenced by tumor formation. The term encompasses cancer localized in tumors, as well as cancer not localized in tumors, such as, for instance, cancer cells that expand from a tumor locally by invasion. Thus, the method has applicability as a local adjuvant therapy for resected cancers as well as a local control of tumor growth.

[0050] The method of the present invention can be combined with other methods of cancer treatment. Examples of such methods include radiation, surgery and chemotherapy. In addition, the method of the present invention can be adapted for non-human mammals, for example, by employing a nonhuman mammalian cell line and a non-human mammalian source of an immunomodulator and viral antigen, as appropriate.

[0051] The present inventive cell line has other uses, other than as described above. For example, the present inventive cell line can be used to characterize a human's immune response to the antigen, e.g., viral antigen, such as an EBV, HPV or KSHV antigen, expressed by the cell line. For example, if the antigen expressed by the cell line is an EBV antigen, the human's immune response to the EBV antigen can be measured before and after administration of the cell line. By comparing the immune responses before and after administration, it is possible to determine whether or not a given human responds immunologically to the antigen of EBV and, if desired, characterize the nature and extent of the response. Tetramer assay and cytokine secretion assay can be used. If the human is to be treated for an EBV-expressing cancer, this information can be used to determine if the human can be treated using the EBV antigen-expressing cell line. This information also can be used to determine how best to administer the EBV antigen-expressing cell line, e.g., what dosage at what frequency. In much the same way, an immunocompromised human can be evaluated to determine suitability for cancer immunotherapy and, if found suitable, the manner of treatment, i.e., dosage and frequency of administration.

[0052] The present inventive cell line also can be used to assess an immune response to a cancer cell vaccine for which the antigen is undefined. For example, the present inventive cell line can be administered to a human in combination with the cancer cell vaccine, and the present inventive cell line can be used as a marker. The immune response to the defined antigen expressed by the present inventive cell line can be used to determine the human's

immune responsiveness, thereby enabling grading of immune responses to the cancer cell vaccine under similar vaccine conditions, for example.

[0053] In view of the teachings set forth herein, Applicants reserve the right to pursue claims to the following embodiments. This reservation is not to be construed as a waiver of the right to pursue claims directed to other embodiments and modifications thereof as described herein.

[0054] A. A human cell line, which lacks MHC-I antigens and MHC-II antigens and which has been modified to comprise and express (i) a nucleotide sequence encoding an immunomodulator and (ii) a nucleotide sequence encoding an antigen of HPV or KSHV.

[0055] B. The human cell line of A, wherein the antigen of HPV is E5, E6 or E7 and the antigen of KSHV is LANA or v-cyclin.

[0056] C. The human cell line of A or B, wherein the immunomodulator is a cytokine, a chemokine or an adjuvant.

[0057] D. The human cell line of C, wherein the cytokine is an inteferon, an interleukin, a tumor necrosis factor, erythropoietin, FLT-3 ligand, macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), or granulocyte-macrophage colony stimulating factor (GM-CSF).

[0058] E. The human cell line of D, wherein the interferon (IFN) is IFNα, IFNβ or IFNγ.

[0059] F. The human cell line of D, wherein the interleukin (IL) is IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8, IL-10 or IL-12.

[0060] G. The human cell line of D, wherein the tumor necrosis factor (TNF) is TNFα or TNFβ.

[0061] H. The human cell line of C, wherein the chemokine is Mip1α, Mip-1β, Mip-3α (Larc), Mip-3β, Rantes, Hcc-1, Mpif-1, Mpif-2, Mcp-1, Mcp-2, Mcp-3, Mcp-4, Mcp-5, Eotaxin, Tarc, Elc, I309, IL-8, Gcp-2 Gro-α, Gro-β, Gro-γ, Nap-2, Ena-78, Gcp-2, Ip-10, Mig, I-Tac, Sdf-1, or Bca-1 (Blc).

[0062] I. The human cell line of C, wherein the adjuvant is a heat shock protein or CpG.

[0063] J. The human cell line of A, wherein the immunomodulator is GM-CSF, and the antigen of HPV is E6 or E7.

[0064] K. The human cell line of A or B, wherein the human cell line that is modified is K562.

[0065] L. The human cell line of C, wherein the human cell line that is modified is K562.

[0066] M. The human cell line of J, wherein the human cell line that is modified is K562.

[0067] N. A method of inducing or stimulating an immune response in a human for an HPV-associated cancer, which method comprises administering to the human the human cell line of A or B in an amount sufficient to induce or stimulate an immune response to the HPV-associated cancer, whereupon an immune response to the HPV-associated cancer is induced or stimulated.

[0068] O. The method of N, wherein the human is female and has cervical cancer.

[0069] P. A method of inducing or stimulating an immune response in a human for an HPV-associated cancer, which method comprises administering to the human the human cell line of C in an amount sufficient to induce or stimulate an immune response to the HPV-associated cancer, whereupon an immune response to the HPV-associated cancer is induced or stimulated.

[0070] Q. The method of P, wherein the human is female and has cervical cancer.

[0071] R. A method of inducing or stimulating an immune response in a human for an HPV-associated cancer, which method comprises administering to the human the human cell line of J in an amount sufficient to induce or stimulate an immune response to the HPV-associated cancer, whereupon an immune response to the HPV-associated cancer is induced or stimulated.

[0072] S. The method of R, wherein the human is female and has cervical cancer.

[0073] T. A method of inducing or stimulating an immune response in a human for an HPV-associated cancer, which method comprises administering to the human the human cell line of K in an amount sufficient to induce or stimulate an immune response to the HPV-associated cancer, whereupon an immune response to the HPV-associated cancer is induced or stimulated.

[0074] U. The method of T, wherein the human is female and has cervical cancer.

[0075] V. A method of inducing or stimulating an immune response in a human for an HPV-associated cancer, which method comprises administering to the human the human cell line of L in an amount sufficient to induce or stimulate an immune response to the HPV-associated cancer, whereupon an immune response to the HPV-associated cancer is induced or stimulated.

[0076] W. The method of V, wherein the human is female and has cervical cancer.

[0077] X. A method of inducing or stimulating an immune response in a human for an HPV-associated cancer, which method comprises administering to the human the human cell line of M in an amount sufficient to induce or stimulate an immune response to the

HPV-associated cancer, whereupon an immune response to the HPV-associated cancer is induced or stimulated.

[0078] Y. The method of X, wherein the human is female and has cervical cancer.

[0079] Z. A composition comprising a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an immunomodulator, and a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of HPV or KSHV.

[0080] AA. A composition comprising an immunomodulator and a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of HPV or KSHV.

[0081] AB. A method of inducing or stimulating an immune response in a human to an HPV-associated or KSHV-associated cancer, which method comprises administering to the human the composition of Z (expressing an antigen of HPV or KSHV, respectively) in an amount sufficient to induce or stimulate an immune response to the HPV-associated or KSHV-associated cancer, whereupon an immune response to the HPV-associated or KSHV-associated cancer is induced or stimulated.

[0082] AC. The method of AB, wherein the human is female and has cervical cancer associated with HPV or the human has Kaposi's sarcoma.

[0083] AD. A method of inducing or stimulating an immune response in a human to an HPV-associated or KSHV-associated cancer, which method comprises administering to the human the composition of AA (expressing an antigen of HPV or KSHV, respectively) in an amount sufficient to induce or stimulate an immune response to the HPV-associated or KSHV-associated cancer, whereupon an immune response to the HPV-associated or KSHV-associated cancer is induced or stimulated.

[0084] AE. The method of AD, wherein the human is female and has cervical cancer associated with HPV or the human has Kaposi's sarcoma.

[0085] AF. A method of inducing or stimulating an immune response in a human to an HPV-associated or KSHV-associated cancer, which method comprises administering to the human a composition comprising a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an immunomodulator, in an amount sufficient to induce or stimulate an immune response, and simultaneously or sequentially, in either order, by the same route or a different route, a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of HPV or KSHV, respectively, in an amount sufficient to induce to stimulate an immune response,

whereupon an immune response to the HPV-associated or KSHV-associated cancer is induced or stimulated.

[0086] AG. The method of AF, wherein the human is female and has cervical cancer associated with HPV or the human has Kaposi's sarcoma.

[0087] AH. A method of inducing or stimulating an immune response in a human to an HPV-associated or KSHV-associated cancer, which method comprises administering to the human an immunomodulator in an amount sufficient to induce to stimulate an immune response, and simultaneously or sequentially, in either order, by the same route or a different route, a human cell line, which lacks MHC-I and MHC-II antigens and which has been modified to comprise and express a nucleotide sequence encoding an antigen of HPV or KSHV, respectively, in an amount sufficient to induce to stimulate an immune response, whereupon an immune response to the HPV-associated or KSHV-associated cancer is induced or stimulated.

[0088] AI. The method of AH, wherein the human is female and has cervical cancer associated with HPV or the human has Kaposi's sarcoma.

EXAMPLES

[0089] Antibody generation and purification, diagnostic platforms, cloning procedures; etc., to the extent that they are not described herein, can be found in references such as the following:

[0090] Sambrook et al., Molecular Cloning, A Laboratory Manual, Vols. I-III, 1989, Cold Spring Harbor Laboratory Press, USA;

[0091] Harlowe and Lane, Antibodies: A Laboratory Manual, 1988 and 1998, Cold Spring Harbor Laboratory Press, USA; and

[0092] Ausubel et al., Current Protocols, 2001, John Wiley and Sons, Inc.

[0093] The following examples serve to illustrate the present invention and are not intended to limit its scope in any way.

EXAMPLE 1

[0094] This example describes the generation of an EBV antigen-specific, GM-CSF-secreting cellular vaccine.

[0095] The allogeneic human erythroleukemia cell line K562 was transfected with a plasmid containing human GM-CSF operably linked to the cytomegaloviral (CMV) promoter, a hygromycin resistance gene, and the Epstein-Barr virus (EBV) nuclear antigen-1 (EBNA1) gene, which is required for the function of the plasmid origin of replication. The plasmid, designated pCEP4-EBNA1/hGM-CSF, is shown in Fig. 1. Hygromycin-

resistant clones were screened for the secretion of GM-CSF. A K562-EBNA1/GM-CSF clone producing over 2,000 ng of GM-CSF/10^6 cells/24 hrs was selected. The high degree of expression of this clone minimizes the number of cells needed for vaccination, while leaving the margin for efficacy well above the threshold of 36 ng/10^6 cells/24 hrs. The K562-EBNA1/GM-CSF clone also was determined to express EBNA1 by Western blot using a monoclonal antibody to EBNA1.

[0096] The K562-EBNA1/GM-CSF clone was transfected with a plasmid expressing latent membrane protein 2 (LMP2) under the control of a CMV promoter. The plasmid, which is designated pcDNA3-LMP2, also contains a neomycin resistance gene and is shown in Fig. 2. G418-resistant clones were analyzed for the expression of LMP2 by immunofluorescent staining with a monoclonal antibody to LMP2. A clone expressing high levels of LMP2 was selected and designated K562-EBNA1/LMP2/GM-CSF. The clone has been determined to express high levels of LMP2 (of expected size) and GM-CSF, as well as EBNA1 RNA and LMP2 RNA. A master cell bank can be generated and a clinical grade vaccine can be generated from the master cell bank. The cells can be irradiated, frozen under controlled conditions, and stored in liquid nitrogen in vials containing 3.3 X 10^7 cells.

EXAMPLE 2

[0097] This example describes an EBV antigen-specific, GM-CSF-secreting cellular vaccine for treating EBV+ tumors.

[0098] Patients with EBV+ Hodgkin's lymphoma (HL) or nasophaaryngeal carcinoma (NPC) at high risk for relapse after primary therapy or those with relapsed or metastatic disease are treated. The patients are given a first vaccination six weeks after the completion of primary therapies, such as chemotherapy, radiation, or a combination thereof. Follow-up vaccinations are given monthly thereafter for a total of four vaccinations, spanning weeks 6 to 18 during immune reconstitution. On the day of vaccination, the cellular vaccine (K562-EBNA1/LMP2/GM-CSF) is removed from the liquid nitrogen storage and rapidly thawed in a 37°C water bath. Viability of the cellular vaccine is assessed by trypan blue exclusion, and the number of viable cells is used for calculation of dosages. The patients are intradermally injected with a total dose of 3.3 x 10^7 cells per vaccination divided into 9 injections of 3.6 x 10^8 cells in a volume of 0.5 ml. Three injections spaced 5 cm apart will be placed on each anterior thigh and the non-dominant arm. Patients are monitored for possible toxicities at the site of vaccination. Systemic toxicities are assessed from paracrine secretion of GM-CSF.

[0099] If desired, the generation and enhancement of LMP2/EBNA1-specific CD4 and CD8 T cell responses to LMP2 and EBNA1 are assessed using a modified IFN-γ ELISPOT

assay, which utilizes dendritic cells infected with recombinant vaccinia virus expressing LMP2 or EBNA1 as a stimulator. This abolishes the need to HLA-type each patient. Briefly, peripheral blood mononuclear cells (PBMCs) from patients are fractioned into CD4 and CD8 cells by a magnetic cell separation (MACS) system. Purified CD4 or CD8 cells are stimulated with dendritic cells transduced with recombinant vaccinia vector encoding LMP2 or EBNA1 in multiscreen hemagglutinin (HA) plates coated with a monoclonal antibody to IFN-γ (capture antibody) for 16-18 hrs. The plates are then washed and stained for IFN-γ with an immunoperoxidase technique. The IFN-γ-positive spots are counted using a stereomicroscope. A tetramer assay also is used for HLA-A2, A11 and A24 patients in order to correlate with results from the IFN-γ ELISPOT assay.

[00100] Patients are compared at baseline and at six months or earlier after completion of vaccination. The baseline cellular response to LMP2 is expected to be less than about 50/million PBMCs. A vaccination is considered to be successful if the cellular response exceeds about 200/million PBMCs.

EXAMPLE 3

[00101] This example describes the generation of an HPV antigen-specific, GM-CSF-secreting cellular vaccine.

[00102] The allogeneic human erythroleukemia cell line K562 was transfected with a plasmid containing human GM-CSF operably linked to the cytomegaloviral (CMV) promoter, a hygromycin resistance gene, and the Epstein-Barr virus (EBV) nuclear antigen-1 (EBNA1) gene, which is required for the function of the plasmid origin of replication. The plasmid, designated pCEP4-EBNA1/hGM-CSF, is shown in Fig. 1. Hygromycin-resistant clones were screened for the secretion of GM-CSF. A K562-EBNA1/GM-CSF clone producing over 2,000 ng of GM-CSF/10^6 cells/24 hrs was selected. The high degree of expression of this clone minimizes the number of cells needed for vaccination, while leaving the margin for efficacy well above the threshold of 36 ng/10^6 cells/24 hrs. The K562-EBNA1/GM-CSF clone also was determined to express EBNA1 by Western blot using a monoclonal antibody to EBNA1.

[00103] The K562-EBNA1/GM-CSF clone is transfected with a plasmid expressing E6 under the control of a CMV promoter. The plasmid, which is designated pcDNA3-E6, also contains a neomycin resistance gene. G418-resistant clones were analyzed for the expression of E6 by immunofluorescent staining with a monoclonal antibody to E6. A clone expressing high levels of E6 was selected and designated K562-EBNA1/E6/GM-CSF. A master cell bank can be generated and a clinical grade vaccine can be generated from the

master cell bank. The cells can be irradiated, frozen under controlled conditions, and stored in liquid nitrogen in vials containing 3.3 X 10^7 cells.

EXAMPLE 4

[00104] This example describes an HPV antigen-specific, GM-CSF-secreting cellular vaccine for treating HPV+ tumors.

[00105] Patients with HPV+ cervical cancer at high risk for relapse after primary therapy or those with relapsed or metastatic disease are treated. The patients are given a first vaccination six weeks after the completion of primary therapies, such as chemotherapy, radiation, or a combination thereof. Follow-up vaccinations are given monthly thereafter for a total of four vaccinations, spanning weeks 6 to 18 during immune reconstitution. On the day of vaccination, the cellular vaccine (K562-EBNA1/E6/GM-CSF) is removed from the liquid nitrogen storage and rapidly thawed in a 37°C water bath. Viability of the cellular vaccine is assessed by trypan blue exclusion, and the number of viable cells is used for calculation of dosages. The patients are intradermally injected with a total dose of 3.3 x 10^7 cells per vaccination divided into 9 injections of 3.6 x 10^8 cells in a volume of 0.5 ml. Three injections spaced 5 cm apart will be placed on each anterior thigh and the non-dominant arm. Patients are monitored for possible toxicities at the site of vaccination. Systemic toxicities are assessed from paracrine secretion of GM-CSF.

[00106] If desired, the generation and enhancement of E6-specific CD4 and CD8 T cell responses to E6 is assessed using a modified IFN-γ ELISPOT assay, which utilizes dendritic cells infected with recombinant vaccinia virus expressing E6 as a stimulator. This abolishes the need to HLA-type each patient. Briefly, PBMCs from patients are fractioned into CD4 and CD8 cells by MACS separation system. Purified CD4 or CD8 cells are stimulated with dendritic cells transduced with recombinant vaccinia vector encoding E6 or EBNA1 in multiscreen HA plates coated with a monoclonal antibody to IFN-γ (capture antibody) for 16-18 hrs. The plates are then washed and stained for IFN-γ with an immunoperoxidase technique. The IFN-γ-positive spots are counted using a stereomicroscope. A tetramer assay also is used for HLA-A2, A11 and A24 patients in order to correlate with results from the IFN-γ ELISPOT assay.

[00107] Patients are compared at baseline and at six months or earlier after completion of vaccination. The baseline cellular response to E6 is expected to be less than about 50/million PBMCs. A vaccination is considered to be successful if the cellular response exceeds about 200/million PBMCs.

EXAMPLE 5

[00108] This example describes the generation of a KSHV antigen-specific, GM-CSF-secreting cellular vaccine.

[00109] The allogeneic human erythroleukemia cell line K562 was transfected with a plasmid containing human GM-CSF operably linked to the cytomegaloviral (CMV) promoter, a hygromycin resistance gene, and the Epstein-Barr virus (EBV) nuclear antigen-1 (EBNA1) gene, which is required for the function of the plasmid origin of replication. The plasmid, designated pCEP4-EBNA1/hGM-CSF, is shown in Fig. 1. Hygromycin-resistant clones were screened for the secretion of GM-CSF. A K562-EBNA1/GM-CSF clone producing over 2,000 ng of GM-CSF/10^6 cells/24 hrs was selected. The high degree of expression of this clone minimizes the number of cells needed for vaccination, while leaving the margin for efficacy well above the threshold of 36 ng/10^6 cells/24 hrs. The K562-EBNA1/GM-CSF clone also was determined to express EBNA1 by Western blot using a monoclonal antibody to EBNA1.

[00110] The K562-EBNA1/GM-CSF clone is transfected with a plasmid expressing LANA under the control of a CMV promoter. The plasmid, which is designated pcDNA3-LANA, also contains a neomycin resistance gene. G418-resistant clones were analyzed for the expression of LANA by immunofluorescent staining with a monoclonal antibody to LANA. A clone expressing high levels of LANA was selected and designated K562-EBNA1/LANA/GM-CSF. A master cell bank can be generated and a clinical grade vaccine can be generated from the master cell bank. The cells can be irradiated, frozen under controlled conditions, and stored in liquid nitrogen in vials containing 3.3 X 10^7 cells.

EXAMPLE 6

[00111] This example describes an KSHV antigen-specific, GM-CSF-secreting cellular vaccine for treating KSHV+ tumors.

[00112] Patients with Kaposi sarcoma at high risk for relapse after primary therapy or those with relapsed or metastatic disease are treated. The patients are given a first vaccination six weeks after the completion of primary therapies, such as chemotherapy, radiation, or a combination thereof. Follow-up vaccinations are given monthly thereafter for a total of four vaccinations, spanning weeks 6 to 18 during immune reconstitution. On the day of vaccination, the cellular vaccine (K562-EBNA1/LANA/GM-CSF) is removed from the liquid nitrogen storage and rapidly thawed in a 37°C water bath. Viability of the cellular vaccine is assessed by trypan blue exclusion, and the number of viable cells is used for calculation of dosages. The patients are intradermally injected with a total dose of 3.3 x 10^7 cells per vaccination divided into 9 injections of 3.6 x 10^8 cells in a volume of 0.5 ml.

Three injections spaced 5 cm apart will be placed on each anterior thigh and the non-dominant arm. Patients are monitored for possible toxicities at the site of vaccination. Systemic toxicities are assessed from paracrine secretion of GM-CSF.

[00113] If desired, the generation and enhancement of LANA-specific CD4 and CD8 T cell responses to LANA is assessed using a modified IFN-γ ELISPOT assay, which utilizes dendritic cells infected with recombinant vaccinia virus expressing LANA as a stimulator. This abolishes the need to HLA-type each patient. Briefly, PBMCs from patients are fractioned into CD4 and CD8 cells by MACS separation system. Purified CD4 or CD8 cells are stimulated with dendritic cells transduced with recombinant vaccinia vector encoding LANA or EBNA1 in multiscreen HA plates coated with a monoclonal antibody to IFN-γ (capture antibody) for 16-18 hrs. The plates are then washed and stained for IFN-γ with an immunoperoxidase technique. The IFN-γ-positive spots are counted using a stereomicroscope. A tetramer assay also is used for HLA-A2, A11 and A24 patients in order to correlate with results from the IFN-γ ELISPOT assay.

[00114] Patients are compared at baseline and at six months or earlier after completion of vaccination. The baseline cellular response to LANA is expected to be less than about 50/million PBMCs. A vaccination is considered to be successful if the cellular response exceeds about 200/million PBMCs.

[00115] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[00116] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise

claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[00117] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.